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(71) Applicant: 1149336 ONTARIO INC. [CA/CA]; 19 Fernwood Road, Toronto, Ontario M6B 3G3 (CA).

(72) Inventor: DRUCKER, Daniel, J.; 19 Fernwood Road, Toronto, Ontario M6V 3G3 (CA).

(74) Agent: AITKEN, David, W.; Osler, Hoskin & Harcourt, Suite 1500, 50 O'Connor Street, Ottawa, Ontario K1P 6L2 (CA).

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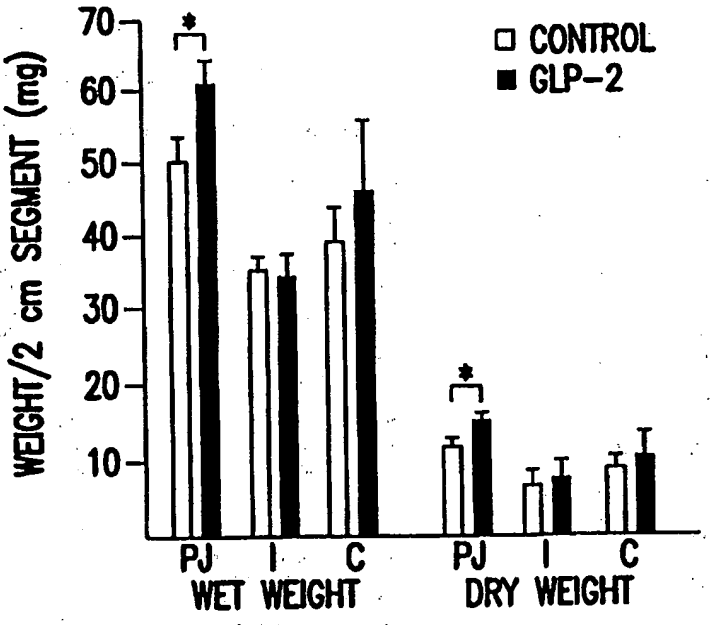
(54) Title: METHODS OF ENHANCING FUNCTIONING OF THE LARGE INTESTINE

(57) Abstract

The invention relates to glucagon–related peptides and their use for the prevention or treatment of disorders involving the large intestine. In particular, it has now been demonstrated that GLP–2 and peptidic agonists of GLP–2 can cause proliferation of the tissue of large intestine. Thus, the invention provides methods of proliferating the large intestine in a subject in need thereof. Further, the methods of the invention are useful to treat or prevent inflammatory conditions of the large intestine, including inflammatory bowel diseases.



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Methods of Enhancing Functioning of the Large Intestine

Field of Invention

This invention relates to glucagon-related peptides and their use for the prevention or treatment of disorders involving the large intestine.

Background of the Invention

Glucagon-like peptide-2 (GLP-2) is a 33 amino acid peptide expressed in a tissue-specific manner from the pleiotropic glucagon gene. GLP-2 shows remarkable homology in terms of amino acid sequence to glucagon and Glucagon-like peptide-1 (GLP-1). Further, different mammalian forms of GLP-2 are highly conserved. For example, the human GLP-2 (hGLP-2) and degu (a south American rodent) GLP-2 differ from rat GLP-2 (rGLP-2) by one and three amino acids respectively. When given exogenously, GLP-2 can produce a marked increase in the proliferation of small intestinal epithelium of the test mice, apparently with no undesirable side effects (Drucker *et al.*, 1996, PNAS:USA, 93:7911-7916). Subsequently it was shown that peptide analogs of native GLP-2 with certain modifications to the peptide sequence possess enhanced intestinotrophic activity at the small intestine (see co-pending application U.S. Serial No. 08/669,791, incorporated herein by reference). Moreover, GLP-2 has also been shown to increase D-Glucose maximal transport rate across the intestinal basolateral membrane [Cheeseman and Tseng, 1996, American Journal of Physiology 271:G477-G482].

Summary of the Invention

The invention is based, in part, on the discovery that GLP-2 receptor agonists act to enhance functioning of the large intestine. It is accordingly a general object of the present invention to exploit GLP-2 receptor agonists for therapeutic and related purposes.

In particular, it has been demonstrated that GLP-2 and peptidic analogs of GLP-2 can cause proliferation of the

tissue of large intestine. Thus, in one aspect the invention provides a method of proliferating the large intestine in a subject in need thereof comprising delivering to the large intestine of the subject a large intestine proliferating amount of GLP-2 or a GLP-2 analog. In a preferred embodiment, the GLP-2 analog is one that is resistant to cleavage by DPP-IV, *e.g.*, human [Gly^2]GLP-2 (referred to herein as [Gly^2]hGLP-2).

More particularly, and according to one aspect of the invention, there is provided a method of treating a subject having an inflammatory condition of the intestine involving the large intestine, wherein GLP-2 or a GLP-2 analog is delivered to the large intestine in an amount capable of ameliorating the inflammation of the large intestine. In a preferred embodiment, the GLP-2 analog is one that is resistant to cleavage by DPP-IV, *e.g.*, [Gly^2]hGLP-2.

In a related aspect of the invention, there is provided a method of treating a subject having an inflamed large intestine comprising the step of delivering to the subject a large intestine inflammation ameliorating amount of GLP-2 or an analog of GLP-2 in a pharmaceutically or veterinarily acceptable carrier. In a further aspect, GLP-2 or an analog of GLP-2 is provided in a pharmaceutically or veterinarily acceptable form in an amount effective to cause proliferation of the large intestine. Preferably, the GLP-2 analog is one that is resistant to cleavage by DPP-IV, *e.g.*, [Gly^2]hGLP-2.

In another aspect, the invention provides a method of prophylactically treating a subject at risk of developing an inflammatory condition of the intestine involving the large intestine comprising the steps of

a) identifying a subject at risk of developing an inflammatory bowel condition involving the large intestine; and

b) administering to the subject an amount of GLP-2 or a GLP-2 analog effective to inhibit onset of the inflammatory condition.

In another aspect of the invention, there is provided a method to identify peptides useful to treat inflammatory conditions involving the large intestine comprising the steps of:

a) obtaining an analog of a vertebrate GLP-2 peptide, the analog having at least one amino acid substitution, deletion, addition, or an amino acid with a blocking group;

b) inducing an inflammatory condition of the intestine involving the large intestine in a test animal;

c) treating the test animal having an induced inflammatory condition of the large intestine, with the analog using a regimen capable of eliciting an amelioration of the inflammatory condition of the large intestine when utilized for human [Gly^2]GLP-2; and

d) determining the effect of the analog on the health status or mortality of the test animal compared with control animals not receiving the peptide or determining the mass of the large intestine of test animals compared to control animals not receiving peptide.

In a related aspect of the invention, there is provided a method useful to identify peptides capable of proliferating the tissue of the large intestine comprising the steps of :

a) obtaining an analog of a vertebrate GLP-2 peptide, the analog having at least one amino acid substitution, deletion, addition, or an amino acid with a blocking group;

b) delivering the analog to the large intestine of the test animal using a regimen capable of proliferating the large intestine when utilized for human [Gly^2]GLP-2; and

c) assessing the increase in the mass or length of the large intestine after completion of the treatment regime.

In another aspect, the invention provides a method for growing large intestine tissue or cells therefrom, which comprises the step of culturing the tissue or cells in a culturing medium supplemented with a growth promoting amount of GLP-2 or a GLP-2 analog.

Brief Description of the Figures

Figure 1 illustrates the change in total protein, and wet and dry weight of the large intestine after treatment with GLP-2. Female CD1 mice were injected with 2.5 μg of rat GLP-2 twice daily for 10 days. In Figure 1A, segments of proximal jejunum (PJ), ileum (I), and colon (C) were analyzed for total protein concentration. Figure 1B presents the wet and dry weight of 2 cm segments of proximal jejunum (PJ), ileum (I), and colon (C).

Figure 2 is a graph of the total body weight over time of mice with experimentally induced colitis that were injected with PBS or with a GLP-2 agonist. The treatment groups are as indicated, and are further described *infra* in Example 2. Figure 2A- treatment groups 1 and 2. Figure 2B- treatment groups 3 and 4. Figure 2C- treatment groups 5 and 6. Figure 2D- treatment groups 7 and 8.

Figure 3 depicts the post-treatment large intestine mass in grams (g) of each treatment group described in Example 2 *infra*.

Figure 4 graphs the post-treatment large intestine length in centimeters (cm) of each treatment group described in Example 2 *infra*.

Detailed Description of the Invention

The invention relates to therapeutic and related uses of GLP-2 and GLP-2 analogs, in particular for the amelioration of medical or veterinary conditions in which functioning of the large intestine is impaired by disease or injury. For example, the method is usefully applied to treat subjects suffering from an inflammatory condition of the large intestine, or subjects who have undergone resection of the large intestine.

As used herein the term "large intestine" means the distal portion of the intestine, extending from its junction with the small intestine to the anus: it comprises the cecum, colon, rectum, and anal canal.

As used herein the term "subject" includes a human or other mammal and including livestock and pets.

As used herein the term "GLP-2 receptor agonist" means any molecule which on binding to the GLP-2 receptor results in activation of the GLP-2 receptor, and includes for example GLP-2 or peptidic analogs of GLP-2. Recently it has been demonstrated that the GLP-2 receptor is a G-protein coupled receptor. Nucleic acid encoding the GLP-2 receptor has been isolated [*see* co-pending applications U.S. Serial No. 08/767,224, filed December 13, 1996 and 08/845,546, filed April 24, 1997, both of which are incorporated herein by reference]. Thus, methods commonly used in this field to identify G-protein coupled receptor agonists may be usefully applied to the GLP-2 receptor. One particularly useful methodology for assessing compounds for GLP-2 receptor agonist activity is disclosed in the above mentioned co-pending applications. Briefly, suitable cells such as COS cells are transformed with GLP-2 receptor encoding nucleic acid such that functional receptor is provided at the cell surface. Thereafter agonist activity of a test compounds can be assessed by contacting transformed cells by the test compound; an increase in the intracellular level of cyclic adenosine monophosphate in response to binding of the test compound to the transformed cells indicates agonist activity.

GLP-2 peptide analogs and selected chemical libraries, may be screened for GLP-2 receptor agonist activity using this approach. Guidance on the types of peptidic analogs that may be usefully employed in this method is given herein and in co-pending applications 08/632,533 and 08/631,273, which are incorporated herein by reference. Moreover, any of the commercially available chemical libraries may be usefully screened for small molecule GLP-2 receptor agonists using high throughput or ultra high throughput screening technology. Peptidic analogs of GLP-2 and small molecule agonists identified as GLP-2 receptor agonists may be screened for therapeutic and related utility to treat

conditions involving the large intestine using the models described herein.

Any subject requiring enhancement of the activity of the large intestine may potentially be a candidate for treatment with a GLP-2 agonist according to the invention. In particular, one group of conditions that may be beneficially treated according to the invention are inflammatory conditions of the intestine involving the large intestine (inflammatory bowel diseases, or "IBD"). Human patients are typically diagnosed as having such a condition after manifesting one or more of the following symptoms: pain in the abdomen, pain with defecation, diarrhea or constipation (best described as a change in the normal bowel "habit"), rectal bleeding, fever, weight loss, anemia, fluid loss leading to dehydration. Visualization using sigmoidoscopy or colonoscopy can be used to confirm the presence of an inflammatory condition of the large intestine. Alternatively, biopsies or a barium enema x-ray can be used to complete the diagnosis. Inflammatory bowel diseases include Crohn's disease and ulcerative colitis, infectious colitis, drug or chemical-induced colitis, diverticulitis, and ischemic colitis. Standards for assessing the severity of such diseases are well known in the art (*see*, for example, Hanuer, 1996, New Eng. J. Med. 334:841-848).

Moreover, subjects identified to be at risk of developing an IBD and subjects in remission from a condition involving inflammation of the large intestine may be beneficially treated prophylactically with a GLP-2 agonist according to the invention to inhibit onset of inflammation of the large intestine. For example, ulcerative colitis and Crohn's disease can be familial diseases, accordingly, linkage studies can identify suspectable individuals (*see*, for example, Hugot *et al.*, 1996, Nature 379:821-823). Further, as it is known that the risk of colitis is increased in persons who have "quit" smoking, GLP-2 could be advantageously administered to such subjects particularly suspectable of developing colitis.

Treatment with GLP-2 agonists has been demonstrated to increase the length of the large intestine. Accordingly, subjects who would benefit from an increase in the length of the large intestine, for example patients who have undergone partial or non-total resection of the large intestine, may be beneficially treated with GLP-2 receptor agonists according to the invention.

A model suitable for determining which analogs of GLP-2 have large intestine proliferation activity are potentially therapeutically useful to treat medical or veterinary conditions of the large intestine is described in Example 1.

Animal models useful for studying inflammatory conditions involving the large intestine are described in the literature. (*See* Elson *et al.*, 1995, Gastroenterology 109:1344-1367; Kim *et al.*, 1992, Scand. J. Gastroenterol. 27:529-537; Dieleman *et al.*, 1994, Gastroenterology 107:1643-1652; Domek *et al.*, 1995, Scand. J. Gastroenterol. 30:1089-1094; Mashimo *et al.*, 1996, Science 274:262-265; Okayasu *et al.*, 1990, Gastroenterology, 98:694-702; Takizawa *et al.*, 1995, Adv. Exp. Med. Biol. 371:1383-1387; and Wells *et al.*, 1990, J. Acquired Imm. Defic. Syndromes 3:361-365.) For example, ulcerative colitis is inducible in test mice using dextran sulphate (Okayasu *et al.*, 1990, *supra*.) and is used herein in Example 2. Typically test mice ingesting 3-10% dextran sulphate in their drinking water show at least one of the following symptoms within 6-10 days: weight loss, rectal bleeding or diarrhea, lethargy, weakness, and decreased movement, eating and drinking. Thus, the animal model described in Example 2 can be used to assess the ability of compounds identified as GLP-2 agonist to ameliorate inflammatory conditions involving the large intestine.

The various vertebrate forms of GLP-2 include, for example, rat GLP-2 and its homologues including ox GLP-2, porcine GLP-2, degu GLP-2, bovine GLP-2, guinea pig GLP-2, hamster GLP-2, human GLP-2, rainbow trout GLP-2, and chicken GLP-2, the sequences of which have been reported by many authors including Buhl *et al.* in J. Biol. Chem., 1988,

263(18):8621, Nishi and Steiner, Mol. Endocrinol., 1990, 4:1192-8, and Irwin and Wong, Mol. Endocrinol., 1995, 9(3):267-77. The sequences reported by these authors is incorporated herein by reference.

Analogs of vertebrate GLP-2 can be generated using standard techniques of peptide chemistry and can be assessed for trophic activity at the large intestine, all according to the guidance provided herein. Particularly preferred analogs of the invention are those based upon the sequence of human GLP-2, as follows:

His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp

wherein one or more amino acid residues are conservatively substituted for another amino acid residue, as long as the analog still maintains its trophic activity at the large intestine as measured by an increase in at least one of the following parameters: large intestine length, protein content or mass.

Conservative substitutions in any naturally occurring GLP-2, preferably the human GLP-2 sequence, are defined as exchanges within any of the following five groups:

I. Ala, Ser, Thr, Pro, Gly
II. Asn, Asp, Glu, Gln
III. His, Arg, Lys
IV. Met, Leu, Ile, Val, Cys
V. Phe, Tyr, Trp.

The invention also encompasses non-conservative substitutions of amino acids in any vertebrate GLP-2 sequence, provided that the non-conservative substitutions occur at amino acid positions known to vary in GLP-2 isolated from different species. Non-conserved residue positions are readily determined by aligning all known vertebrate GLP-2 sequences. For example, Buhl *et al.*, J. Biol. Chem., 1988, 263(18):8621, compared the sequences of human, porcine, rat, hamster, guinea pig, and bovine GLP-2's, and found that positions 13, 16, 19, 27 and 28 were non-conserved (position numbers refer

to the analogous position in the human GLP-2 sequence). Nishi and Steiner, Mol. Endocrinol., 1990, 4:1192-8, found that an additional position within the sequence encoding GLP-2, residue 20 in the above human sequence, also varied in degu, a rodent species indigenous to South America. Thus, under this standard, the amino acid positions which vary in mammals and which preferable may be substituted with non-conservative residues are positions 13, 16, 19, 20, 27 and 28. The additional amino acid residues which vary in vertebrates and which also may be substituted with non-conserved residues occur at positions 2, 5, 7, 8, 9, 10, 12, 17, 21, 22, 23, 24, 26, 29, 30, 31, 32 and 33.

Alternatively, non-conservative substitutions may be made at any position in which alanine-scanning mutagenesis reveals some tolerance for mutation in that substitution of an amino acid residue with alanine does not destroy all intestinotrophic activity at the large intestine. The technique of alanine scanning mutagenesis is described by Cunningham and Wells, Science, 1989, 244:1081, and incorporated herein by reference in its entirety. Since most GLP-2 sequences consist of only approximately 33 amino acids (and in human GLP-2 alanine already occurs at four positions), one of skill in the are could easily test an alanine analogue at each remaining position for intestinotrophic effect, as taught in the examples below.

In specific embodiments of the invention, the GLP-2 peptide is selected from

1) rat GLP-2 having the sequence illustrated below:

His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Thr-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp;

2) human GLP-2, the Thr^{19} to Ala^{19} equivalent of rat GLP-2, illustrated below

His-Ala-Asp-Gly-Ser-Phe-Ser-Asp-Glu-Met-Asn-Thr-Ile-Leu-Asp-Asn-Leu-Ala-Ala-Arg-Asp-Phe-Ile-Asn-Trp-Leu-Ile-Gln-Thr-Lys-Ile-Thr-Asp;

3) human [Gly^2]GLP-2 (human GLP-2 wherein the alanine at position 2 is replaced by a glycine);

4) GLP-2's, and GLP-2 analogs, which incorporate an N-terminal blocking group and/or an N-terminal extension such as Arg or Arg-Arg; and/or incorporate a C-terminal blocking group and/or a C-terminal extension such as Arg or Arg-Arg.

Guidance on particular analogs and variants of GLP-2 that may be usefully employed in the present invention, and guidance on how to produce others, is provided in co-pending applications U.S. Serial Nos. 08/669,790 and 08/669,791, both filed on June 28, 1996, the disclosures of which are incorporated herein by reference. Briefly, any substitution, addition or deletion of GLP-2 that does not destroy the activity of GLP-2 may be usefully employed in this invention. In preferred embodiments the GLP-2 analogs are at least as active as native human GLP-2. In the most preferred embodiments, the GLP-2 analog has enhanced activity compared with native human GLP-2. For example, such analogs may exhibit enhanced serum stability, enhanced receptor binding and enhanced signal transducing activity. Other modifications to GLP-2 and GLP-2 analogs that may usefully be employed in this invention are those which render the molecule resistant to oxidation.

In a preferred embodiment of the invention, the GLP-2 analog is produced by the alteration of native GLP-2 to confer DPP-IV resistance, *e.g.*, by substituting a Gly for Ala at position two. The DPP-IV-resistant class of GLP-2 analogs possess particularly advantageous properties. Mammalian GLP-2 species have been found to be sensitive to cleavage by DPP-IV enzyme. This sensitivity to DPP-IV is the result of the recognition sequence Ala^2 Asp^3 found in all mammalian forms of GLP-2. In a preferred embodiment, the GLP-2 analogs are members of a class of GLP-2 analogs which incorporate at position 2 and or position 3 a replacement amino acid which confers on the GLP-2 analog relative resistance to DPP-IV mediated cleavage, as determined by any convenient <u>in vitro</u> or <u>in vivo</u> assessment technique that is able to detect the

presence of GLP-2 digestion products. A DPP-IV resistant GLP-2 analog is revealed as that GLP-2 analog which is processed or degraded at a rate that is measurably slower than the rate at which human GLP-2 is processed or degraded, under the same conditions.

The position two class of GLP-2 analogs is preferred herein. These Ala^2-substituted GLP-2 analogs can incorporate at position two a structurally wide variety of Ala-replacement amino acids to achieve relative resistance to DPP-IV digestion. A similarly wide variety of Ala-replacement amino acids allow also for the retention by the analog of intestinotrophic activity. For purposes of identifying those DPP-IV-resistant position two analogs that also retain intestinotrophic activity, the position two analogs showing DPP-IV resistance are screened in an assay of intestinotrophic activity. Such an assay is described in co-pending application U.S. Serial No. 08/669,791, filed on June 28, 1996, the disclosure of which is incorporated herein by reference.

In embodiments of the present invention, the Ala^2 replacements include stereoisomers of amino isomers that would otherwise be substrates for DPP-IV, for example D-Ala, D-HPr and D-Pro; naturally occurring amino acids other than Ala, HPr and Pro which provide a basic or uncharged side chain, for example, Glu, Lys, Arg, Leu, Ile, Gly and Val. Particularly preferred GLP-2 analogs include [$D\text{-}Ala^2$]rGLP-2, [Gly^2]rGLP-2, [Val^2]rGLP-2 and [Gly^2]hGLP-2.

Further, a large number of agonist GLP-2 peptides that are described in PCT Application PCT/CA97/00252, filed April 11, 1997, incorporated in its entirety by reference herein, may also be used in the methods of the invention.

The "blocking groups" represented by R1 and R2 are chemical groups that are routinely used in the art of peptide chemistry to confer biochemical stability and resistance to digestion by exopeptidase. Suitable N-terminal protecting groups include, for example, C_{1-5}alkanoyl groups such as acetyl. Also suitable as N-terminal protecting groups are

amino acid analogues lacking the amino function. Suitable C-terminal protecting groups include groups which form ketones or amides at the carbon atom of the C-terminal carboxyl, or groups which form esters at the oxygen atom of the carboxyl. Ketone and ester-forming groups include alkyl groups, particularly branched or unbranched C_{1-5} alkyl groups, *e.g.*, methyl, ethyl, and propyl groups, while amide-forming groups include amino functions such as primary amine, or alkylamino functions, *e.g.*, mono-C_{1-5}alkylamino and di-C_{1-5}alkylamino groups such as methylamino, ethylamino, dimethylamino, diethylamino, methylethylamino and the like. Amino acid analogues are also suitable for protecting the C-terminal end of the present compounds, for example, decarboxylated amino acid analogues such as agmatine.

The particular form of GLP-2 selected for promoting the growth of large intestinal tissue can be prepared by a variety of techniques well known for generating peptide products. Vertebrate forms of GLP-2 can of course be obtained by extraction from the natural source, using an appropriate combination of protein isolation techniques. As described by Buhl *et al.*, *supra*, porcine GLP-2 isolation and purification is achieved from acid-ethanol extracts of ileal mucosa by a combination of size selection and HPLC-based fractionation, with the aid of antibody raised against synthetic proglucagon 126-159, to monitor work-up. As an alternative to GLP-2 extraction, those forms of GLP-2 that incorporate only L-amino acids, whether vertebrate GLP-2 or analogs thereof, can be produced in commercial quantities by application of recombinant DNA technology. For this purpose, DNA coding for the desired GLP-2 or GLP-2 analog is incorporated into an expression vector and transformed into a microbial, *e.g.*, yeast, or other cellular host, which is then cultured under conditions appropriate for GLP-2 expression. A variety of gene expression systems have been adapted for this purpose, and typically drive expression of the desired gene from expression controls used naturally by the chosen host. Because GLP-2 does not require post translational

glycosylation for its activity, its production may most conveniently be achieved in bacterial hosts such as E. coli. For such production, DNA coding for the selected GLP-2 peptide may usefully be placed under expression controls of the lac, trp or PL genes of E. coli. As an alternative to expression of DNA coding for the GLP-2 per se, the host can be adapted to express GLP-2 peptide as a fusion protein in which the GLP-2 is linked releasable to a carrier protein that facilitates isolation and stability of the expression product.

In an approach universally applicable to the production of a selected GLP-2 or GLP-2 analog, and one used necessarily to produce GLP-2 peptides that incorporate non-genetically encoded amino acids and N- and C-terminally derivatized forms, the well established techniques of automated peptide synthesis are employed, general descriptions of which appear, for example, in J. M. Stewart and J. D. Young, Solid Phase Peptide Synthesis, 2nd Edition, 1984 Pierce Chemical Company, Rockford, Illinois; and in M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis, 1984, Springer-Verlag, New York; Applied Biosystems 430A Users Manual, 1987, ABI Inc. Foster City, California. In these techniques, GLP-2 peptide is grown from its C-terminal, resin-conjugated residue by the sequential addition of appropriately protected amino acids, using either the Fmoc or tBoc protocols, as described for instance by Orskov *et al.*, Febs Letters, 1989, 247(2):193-196.

For the incorporation of N- and/or C- blocking groups, protocols conventional to solid phase peptide synthesis methods can also be applied. For incorporation of C-terminal blocking groups, for example, synthesis of the desired peptide is typically performed using, as solid phase, a supporting resin that has been chemically modified so that cleavage from the resin results in a GLP-2 peptide having the desired C-terminal blocking group. To provide peptides in which the C-terminus bears a primary amino blocking group, for instance, synthesis is performed using a

p-methylbenzhydrylamine (MBHA) resin so that, when peptide synthesis is completed, treatment with hydrofluoric acid releases the desired C-terminally amidated peptide. Similarly, incorporation of an N-methylamine blocking group at the C-terminus is achieved using N-methylaminoethyl-derivatized DVB resin, which upon HF treatment releases peptide bearing an N-methylamidated C-terminus. Protection of the C-terminus by esterification can also be achieved using conventional procedures. This entails use of resin/blocking group combination that permits release of side-chain protected peptide from the resin, to allow for subsequent reaction with the desired alcohol, to form the ester function. FMOC protecting groups, in combination with DVB resin derivatized with methoxyalkoxybenxyl alcohol or equivalent linker, can be used for this purpose, with cleavage from the support being effected by TFA in dichloromethane. Esterification of the suitably activated carboxyl function, *e.g.*, with DCC, can then proceed by addition of the desired alcohol, followed by deprotection and isolation of the esterified GLP-2 peptide.

Incorporation of N-terminal blocking groups can be achieved while the synthesized GLP-2 peptide is still attached to the resin, for instance by treatment with suitable anhydride and nitrile. To incorporate an acetyl blocking group at the N-terminus, for instance, the resin-coupled peptide can be treated with 20% acetic anhydride in acetonitrile. The N-blocked GLP-2 peptide can then be cleaved from the resin, deprotected and subsequently isolated.

Once the desired GLP-2 peptide has been synthesized, cleaved from the resin and fully deprotected, the peptide is then purified to ensure the recovery of a single oligopeptide having the selected amino acid sequence. Purification can be achieved using any of the standard approaches, which include reversed-phase high-pressure liquid chromatography (RP-HPLC) on alkylated silica columns, *e.g.*, C_4-, C_8-, or C_{18}- silica. Such column fractionation is generally accomplished by

running linear gradients, *e.g.*, 10 - 90%, of increasing % organic solvent, *e.g.*, acetonitrile, in aqueous buffer, usually containing a small amount (*e.g.*, 0.1%) of pairing agent such as TFA or TEA. Alternatively, ion-exchange HPLC can be employed to separate peptide species on the basis of their charge characteristics. Column fractions are collected, and those containing peptide of the desired/required purity are optionally pooled. In one embodiment of the invention, the GLP-2 peptide is then treated in the established manner to exchange the cleavage acid (*e.g.*, TFA) with a pharmaceutically acceptable acid, such as acetic, hydrochloric, phosphoric, maleic, tartaric, succinic and the like, to generate a pharmaceutically acceptable acid addition salt of the peptide.

For administration to patients, the GLP-2 peptide or its salt is provided, in one aspect of the invention, in pharmaceutically acceptable form, *e.g.*, as a preparation that is sterile-filtered, *e.g.*, through a 0.22μ filter, and substantially pyrogen-free. Desirably, the GLP-2 peptide to be formulated migrates as a single or individualized peak on HPLC, exhibits uniform and authentic amino acid composition and sequence upon analysis thereof, and otherwise meets standards set by the various national bodies which regulate quality of pharmaceutical products.

For therapeutic use, the chosen GLP-2 or GLP-2 analog is formulated with a carrier that is pharmaceutically acceptable and is appropriate for administering the peptide to the subject by the chosen route of administration so as to deliver the peptide to the large intestine. Suitable pharmaceutically acceptable carriers are those used conventionally with peptide-based drugs, such as diluents, excipients and the like. Reference may be made to "Remington's Pharmaceutical Sciences", 17th Ed., Mack Publishing Company, Easton, Penn., 1985, for guidance on drug formulations generally. In one embodiment of the invention, the compounds are formulated for administration by infusion, *e.g.*, when used as liquid nutritional supplements for

patients on total parenteral nutrition therapy, or by injection, *e.g.*, sub-cutaneously, intramuscularly or intravenously, and are accordingly utilized as aqueous solutions in sterile and pyrogen-free form and optionally buffered to physiologically tolerable pH, *e.g.*, a slightly acidic or physiological pH. Thus, the compounds may be administered in a vehicle such as distilled water or, more desirably, in saline, phosphate buffered saline or 5% dextrose solution. Water solubility of the GLP-2 or GLP-2 analog may be enhanced, if desired, by incorporating a solubility enhancer, such as acetic acid or sodium hydroxide.

The aqueous carrier or vehicle can be supplemented for use as injectables with an amount of gelatin effective to achieve the depot effect are expected to lie in the range from 10-20%. Alternative gelling agents, such as hyaluronic acid, may also be useful as depoting agents (also veterinary applications).

As an alternative to injectable formulations, the GLP-2 or GLP-2 analog may be formulated for administration to patients and delivery to the large intestine by other routes. Oral dosage forms, such as tablets, capsules and the like, can be formulated in accordance with standard pharmaceutical practice.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

The GLP-2's and GLP-2 analogs of the invention may also be formulated as a slow release implantation device for extended and sustained administration of GLP-2. Examples of such sustained release formulations include composites of bio-compatible polymers, such as poly(lactic acid), poly(lactic-co-glycolic acid), methylcellulose, hyaluronic acid, collagen, and the like. The structure, selection and use of degradable polymers in drug delivery vehicles have been reviewed in several publications, including, A. Domb *et al.*, Polymers for Advanced Technologies 3:279-292 (1992).

Additional guidance in selecting and using polymers in pharmaceutical formulations can be found in the text by M. Chasin and R. Langer (eds.), "Biodegradable Polymers as Drug Delivery Systems", Vol. 45 of "Drugs and the Pharmaceutical Sciences", M. Dekker, New York, 1990. Liposomes may also be used to provide for the sustained release of a GLP-2 or GLP-2 analog. Details concerning how to use and make liposomal formulations of drugs of interest can be found in, among other places, U.S. Pat. No. 4,921,706; U.S. Pat. No. 5,008,050; U.S. Pat. No. 4,921,706; U.S. Pat. No. 4,927,637; U.S. Pat. No. 4,452,747; U.S. Pat. No. 4,016,100; U.S. Pat. No. 4,311,712; U.S. Pat. No. 4,370,349; U.S. Pat. No. 4,372,949; U.S. Pat. No. 4,529,561; U.S. Pat. No. 5,009,956; U.S. Pat No. 4,725,442; U.S. Pat. No. 4,737,323; U.S. Pat. No. 4,920,016. Sustained release formulations are of particular interest when it is desirable to provide a high local concentration of a GLP-2 or GLP-2 analog, *e.g.*, near or at the large intestine to promote large intestine growth in colitis *etc.*

The GLP-2's and GLP-2 analogs of the invention may also be administered to a subject in admixture with at least one other peptide hormone selected from the group consisting of IGF-1, IGF-2 and GH. Such combinations have been shown to have synergistic effects on the growth of the large bowel (*see* U.S. Patent Application Serial No. 08/763,177, filed December 10, 1996, incorporated herein by reference). However, as it has been found that GLP-2 alone can stimulate large intestine growth and ameliorate inflammatory conditions of the large bowel, the invention encompasses GLP-2 and GLP-2 analog formulations which do not contain IGF-1, IGF-2 or GH peptide hormones.

For use in stimulating growth of the large intestine in a mammal including a human, the present invention provides in one of its aspects a package, in the form of a sterile-filled vial or ampoule, that contains a tissue growth promoting amount of the GLP-2 or GLP-2 analog, in either unit dose or multi-dose amounts, wherein the package incorporates a label

instructing use of its contents for the promotion of such growth. In one embodiment of the invention, the package contains the GLP-2 or GLP-2 analog and the desired carrier, as an administration-ready formulation. Alternatively, and according to another embodiment of the invention, the package provides the GLP-2 or GLP-2 analog in a form, such as a lyophilized form, suitable for reconstitution in a suitable carrier, such as phosphate-buffered saline.

In one embodiment, the package is a sterile-filled vial or ampoule containing an injectable solution which comprises an effective, large intestine proliferating amount of GLP-2 or GLP-2 analog dissolved in an aqueous vehicle.

According to the present invention, the GLP-2 or GLP-2 analog is administered to treat patients that would benefit from growth of the tissue of the large intestine. In general, patients who would benefit from either increased large intestinal mass and consequent increased large intestine mucosal function are candidates for treatment with GLP-2 or GLP-2 analog. Particular conditions that may be treated with GLP-2 include the various forms of inflammatory bowel disease including colitis and IBD, as well as patients who have undergone partial or sub-total resection of the large intestine. The therapeutic efficacy of the GLP-2 treatment may be monitored by: subjective improvement in abdominal pain, diarrhea or rectal bleeding; weight gain; normalization of hemoglobin or white blood cell count and sedimentation rate; improved appearance of the intestine on colonoscopy or sigmoidoscopy, improvement of intestine function as assessed radiologically by barium enema; histological improvement as assessed by specimen biopsy; and by amelioration (reduction or elimination) of the symptoms associated with these conditions. For example, GLP-2 or GLP-2 analog is administrated to a patient with an inflammatory condition involving the large intestine in an amount sufficient to ameliorate the intestinal discomfort, bleeding and diarrhea caused by the condition. Additionally, GLP-2 or

GLP-2 analog may be administered to patients who are identified as being at risk of developing IBD.

The therapeutic dosing and regimen most appropriate for patient treatment will of course vary with the disease or condition to be treated, and according to the patient's weight and other parameters. The results presented hereinbelow demonstrate that a dose of GLP-2 or GLP-2 analog equivalent to about 1 mg/kg (or less, see below) administered twice daily over 10 days can ameliorate inflammatory conditions of the large intestine. It is expected that much smaller doses, *e.g.*, in the μg/kg range, and shorter or longer duration or frequency of treatment, will also produce therapeutically useful results, i.e., a statistically significant increase particularly in large intestine mass. The dosage sizes and dosing regimen most appropriate for human use are guided by the results herein presented, and can be confirmed in properly designed clinical trials.

An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Primary among these is the amount of GLP-2 normally circulating in the plasma, which is on the order of 151 pmol/mL in the resting state, rising to 225 pmol/mL after nutrient ingestion for healthy adult humans (Orskov, C. and Holst, J. J., 1987, Scand. J. Clin. Lab. Invest. 47:165). Additional factors include the size of the patient, the age of the patient, the general condition of the patient, the particular disease being treated, the severity of the disease, the presence of other drugs in the patient, the *in vivo* activity of the GLP-2 peptide and the like. The trial dosages would be chosen after consideration of the results of animal studies and the clinical literature. It will be appreciated by the person of ordinary skill in the art that information such as binding constants and Ki derived

from *in vitro* GLP-2 binding competition assays may also be used in calculating dosages.

A typical human dose of a GLP-2 peptide would be from about 10 μg/kg body weight/day to about 10 mg/kg/day, preferably from about 50 μg/kg/day to about 5 mg/kg/day, and most preferably about 100 μg/kg/day to 1 mg/kg/day.

In another of its aspects, the invention provides for the treatment of patient candidates as just identified using implanted cells that have either been conditioned *in vitro* or *in vivo* by prior incubation or treatment with GLP-2 or GLP-2 analog, or have been engineered genetically to produce it. Conditioning of the cells *ex vivo* can be achieved simply by growing the cells or tissue to be transplanted in a medium that has been supplemented with a growth-promoting amount of the GLP-2 or GLP-2 analog and is otherwise appropriate for culturing of those cells. The cells can, after an appropriate conditioning period, then be implanted either directly into the patient or can be encapsulated using established cell encapsulation technology, and then implanted.

Yet another aspect of the invention encompasses treating animals *in vivo* with GLP-2 peptides in order to promote the growth of large intestine tissue. After subsequent enlargement of the large intestine these tissues may then be used in a xenotransplantation procedure. Such GLP-2 peptide treatment can be advantageous prior to xenotransplantation of the tissue from a non-human animal to a human because the size of the transplanted organ or tissue often limits the success of this procedure. For example, a porcine donor animal may be treated with GLP-2 peptide in order to increase large intestine size prior to xenotransplantation of the porcine large intestine tissue into a human in need of this organ.

Alternatively, the cells to be implanted can be raised *in vitro* from a cell that has been engineered genetically to express or to over-express either the glucagon gene or, more directly, DNA coding solely for GLP-2. The sequence of such

DNA can readily be determined from the amino acid sequence of the selected GLP-2, with the limitation that only GLP-2 forms containing genetically encoded amino acids can be produced in this manner. Various viral vectors, suitable for introduction of genetic information into human cells, can be employed and will incorporate the GLP-2-encoding DNA under expression controls functional in the host cells. Once altered genetically, the engineered cells can then be implanted using procedures established in the art. (*See*, for example, Drucker *et al.*, 1996, PNAS:USA, 93:7911-7916.)

The invention having been described, the following examples are offered by way of illustration and not limitation.

Example 1

In this experiment, the effect of GLP-2 on both small and large intestinal mass, as well as the relative contribution of water to the increment in intestine weight, was examined.

Female CD1 mice (6 weeks old) were treated with rat GLP-2, 2.5 μg twice daily (subcutaneously) for 10 days. Following treatment, mice were fasted 24 hours, anesthetized with CO_2 and sacrificed. The small and large intestine was removed from the peritoneal cavity (from pylorus to cecum), cleaned, weighed and measured. For comparative purposes, 2 cm segments of proximal jejunum, ileum and colon were obtained from each animal from the identical anatomical positions (as measured from the pylorus and cecum) and analyzed for protein content, wet weight and dry weight (expressed as mean ± S.E.M in mg/ 2 cm segment).

For protein content, two 2 cm segments from the proximal jejunum, ileum and colon were removed, placed in 13 ml tubes containing 2 ml of PBS, homogenized for 20 seconds in a Brinkmann Homogenizer and placed on ice. The tubes were centrifuged for 5 min. at 1000 g and 100 μl aliquots of supernatant homogenate removed for determination of protein content using the modified Bradford method.

To measure wet and dry weight, 2 cm intestinal segments were weighed, placed in culture tubes, reweighed, then freeze-dried overnight in a Fast-Freeze flask (Labconco) and reweighed.

Results are shown in Figure 1A and 1B. Both the wet and dry weights of intestinal segments from the proximal jejunum were significantly increased following treatment with GLP-2 ($p<.05$). The weights of the segments obtained from the colon were slightly, but consistently, increased after GLP-2 treatment. A statistically significant increase in protein content was observed in both the jejunum and colon of GLP-2-treated mice as compared to PBS-treated mice.

Example 2

This experiment was designed to test the activity of GLP-2 in ameliorating disease symptoms in an animal model of colitis. A 5% dextran sulfate solution was used to induce ulcerative colitis in test mice (Okayasu *et al.*, 1990, 98:694-702).

Forty female CD1 mice (six-weeks old and approximately 25 g, obtained from Charles River) were used in the experiment. On day 1, the mice were weighed using a Mettler PJ300 scale and randomly allocated to 1 of eight treatment groups. The groups, each consisting of 5 mice housed together, were treated as follows:

GROUPS 1 and 2 - CONTROLS

These two groups were fed normal drinking water.

Group 1) Ten days of injections (PBS), day 7 to day 17, animals sacrificed day 17.

Group 2) Ten days of injections human (Gly 2) GLP-2 (2.5 μg bid), day 7 to day 17, animals sacrificed day 17.

GROUPS 3 and 4 - TEST GROUP

Drinking water given to these groups contained 5% Dextran sulfate (DS).

Group 3) DS given from day 7 to day 17, PBS injections given from day 7 to day 17, animals sacrificed day 17.

Group 4) DS given from day 7 to day 17, human[Gly^2] GLP-2 (2.5 μg bid) injections given day 7 to day 17, animals sacrificed day 17.

GROUPS 5 and 6 - TEST GROUP

Drinking water contained 5% Dextran sulfate day 2 to day 9, followed by 4 days of normal drinking water. Ten days of injections were started day 4.

Group 5) DS from day 2 to day 9, switch to normal water day 10 to day 14, injections (PBS) from day 4 to day 14, animals sacrificed day 14.

Group 6) DS from day 2 to day 9, switch to normal water day 10 to day 14, human [Gly^2]GLP-2 (2.5 μg bid) injections given day 4 to day 14, animals sacrificed day 14.

GROUPS 7 and 8 - TEST GROUP

Animals were given eight days of drinking water containing 5% Dextran sulfate, followed by 4 days normal water. Ten days of injections started 4 days after the start of the 5% Dextran sulfate regimen.

Group 7) DS from day 2 to day 9, switch to normal water day 10, PBS injections given from day 6 to day 16, animals sacrificed day 16.

Group 8) DS from day 2 to day 9, switch to normal water day 10, human [Gly^2]GLP-2 (2.5 μg bid) day 6 to day 16, animals sacrificed day 16.

Dextran sulfate (USB, Cleveland, Ohio, MW 40,000-50,000) was dissolved into double distilled water, and placed into the water bottles. Water bottles were topped up daily. GLP-2 peptide was reconstituted and aliquoted on the day prior to the first day of injection and kept in a -20 C freezer. Human [Gly^2]GLP-2 (5mg/mL) was reconstituted in PBS. One microliter of 5N NaOH was required to dissolve the peptide in a volume of 1 ml. The injection volume of 0.5ml was constant throughout the experiment using 1/2 cc U-100 Insulin Syringes Becton Dickinson and Company, NJ). Control mice (cage 1) received the same volume (0.5ml) of phosphate buffered saline (PBS - 137mM NaCl, 2.7mM KCl, 4.3mM $Na_2HPO_4.7H_2O$, 1.4mM KH_2PO_4, pH 7.3).

Subcutaneous injections in the right hind quarter began on various days depending on the treatment conditions given above. All injections were twice a day, at 8 am and 6pm. Mice in cages 1 and 2 were fasted on the day prior to sacrifice. Body weight measurements were made on day 1, day 7, day 12 and the day of sacrifice. The weight and length of the large intestine of each animal was measured after sacrifice.

Average body weights for each group during the course of the experiment are presented graphically in Figure 2. The results for groups 3 and 4 demonstrate that test animals given injections of an analog of GLP-2 maintain body weight better through the course of the experiment and have significantly heavier large intestines at sacrifice than mice injected with PBS.

Figure 3 illustrates the effects of treatment on post-treatment large intestinal mass. As described in Example 1, administration of GLP-2 agonist causes an increase in large intestinal mass (compare groups 1 and 2). Similarly, when GLP-2 agonist is provided at the same time as induction of

colitis by dextran sulfate, GLP-2 agonist ameliorates the severe morbidity, dehydration, and reduction in mass of the large intestine caused by extended periods of induced colitis (compare groups 3 and 4). However, shorter periods of chemically induced colitis caused a generalized inflammation and consequent increase in weight of the large bowel (groups 5 and 7).

GLP-2 agonist also caused a significant increase in large intestine length, as shown in Figure 4 (compare groups 1 and 2). This effect was also exhibited, although to a lesser degree, during experimentally induced colitis (compare groups 3 and 4).

Thus, the above results demonstrate that GLP-2 agonist not only has an ameliorating effect on the symptoms of inflammatory conditions involving the large intestine (when provided at the same time as intestine inflammation is induced), but also causes an increase in both large intestinal weight and length.

Example 3

In the following experiment, GLP-2 agonist was shown to decrease mortality in mice exposed to high levels of colitis-inducing dextran sulphate.

Two groups of mice, 5-6 weeks of age, (10 mice per group) were given ad libitum access to drinking water supplemented with 10% dextran sulfate (same protocol as in Example 2, only increased concentration of dextran sulfate). Mice were also treated with 2 subcutaneous injections per day (0.5 ml) of either saline, or [GLY^2]hGLP-2, 2.5 μg per injection. After 9 days, the experiment was stopped.

Only 3 of 10 mice in the saline-treated group survived, whereas 7 of 10 mice in the GLP-2 agonist-treated group survived. Accordingly, GLP-2-treated mice were better resistant to the effects of dextran sulphate, and had decreased mortality, than mice treated with PBS alone.

EQUIVALENTS

The foregoing written specification is sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above-described means for carrying out the invention which are obvious to those skilled in the field of molecular biology, medicine or related fields are intended to be within the scope of the following claims.

What is Claimed Is:

1. A method for treating a subject to enhance functioning of the large intestine, comprising the step of delivering to the large intestine of the subject a GLP-2 receptor agonist in an amount effective to enhance functioning of the large intestine.

2. A method of treating a subject to proliferate the tissue of the large intestine, comprising the step of delivering to the large intestine of the subject GLP-2 or a peptidic analog of GLP-2, in an amount effective to proliferate the tissue of the large intestine.

3. A method according to claim 2, wherein the subject is suffering from an inflammatory condition involving the large intestine.

4. A method according to claim 3, wherein the inflammatory condition involving the large intestine is selected from the group comprising Crohn's disease, ulcerative colitis, infectious colitis, drug or chemical-induced colitis, diverticulitis, and ischemic colitis.

5. A method according to claim 2, wherein the subject has undergone partial or subtotal resection of the large intestine.

6. A method according to claim 2, wherein the subject is a human.

7. A method according to claim 6, wherein the analog of GLP-2 has enhanced large intestine cell proliferating activity relative to native rat GLP-2.

8. A method according to claim 7, wherein the analog of GLP-2 is resistant to cleavage by DPP-IV.

9. A method according to claim 8, wherein the analog of GLP-2 is selected from the group consisting of [D-Ala^2]rGLP-2, [Gly^2]rGLP-2, [Val^2]rGLP-2, and [Gly^2]hGLP-2.

10. A method according to claim 8, wherein the analog of GLP-2 is [Gly^2]hGLP-2.

11. A method according to claim 8, wherein the analog of GLP-2 is delivered to the large intestine by oral, subcutaneous, or rectal administration.

12. A method to identify peptides useful to treat inflammatory conditions involving the large intestine, comprising the steps of:

a) obtaining an analog of a vertebrate GLP-2 peptide, the analog having at least one amino acid substitution, deletion, addition, or an amino acid with a blocking group;

b) inducing an inflammatory condition of the intestine involving the large intestine in a test animal;

c) treating the test animal having an induced inflammatory condition of the large intestine, with the analog using a regimen capable of eliciting an amelioration of the inflammatory condition of the large intestine when utilized for native GLP-2; and

d) determining the effect of the analog on the health status or mortality of the test animal compared with control animals not receiving the peptide or determining the effect of the analog on the weight of the large intestine of test animals compared to control animals not receiving peptide.

13. A method useful to identify peptides capable of proliferating tissue of the large intestine, comprising the steps of

a) obtaining an analog of a vertebrate GLP-2 peptide, the analog having at least one amino acid substitution, deletion, addition, or an amino acid with a blocking group;

b) delivering the analog to the large intestine of the test animal using a regimen capable of eliciting proliferation of the large intestine when utilized for native GLP-2; and

c) assessing the increase in the mass or length of the large intestine after completion of the treatment regimen.

14. A method of prophylactically treating a subject at risk of developing an inflammatory condition of the intestine involving inflammation of the large intestine, the method comprising:

a) identifying a subject at risk of developing an inflammatory bowel condition involving the large intestine; and

b) administering to the subject an amount of GLP-2 or a GLP-2 analog demonstrates to inhibit onset of the inflammatory condition.

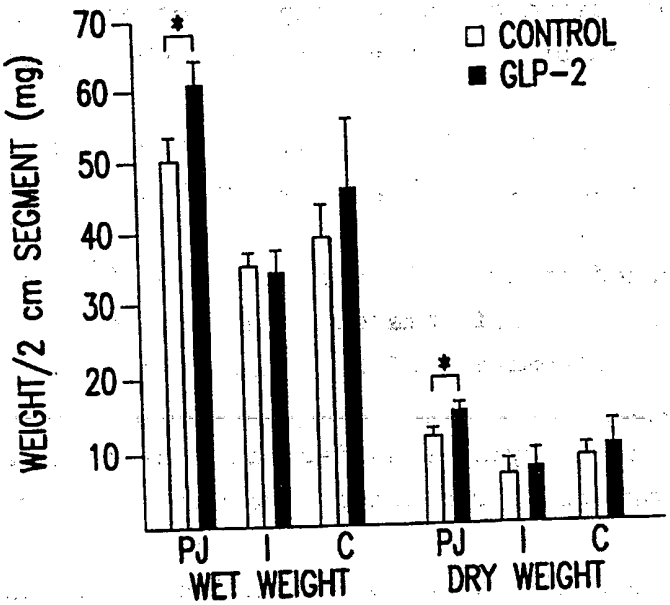
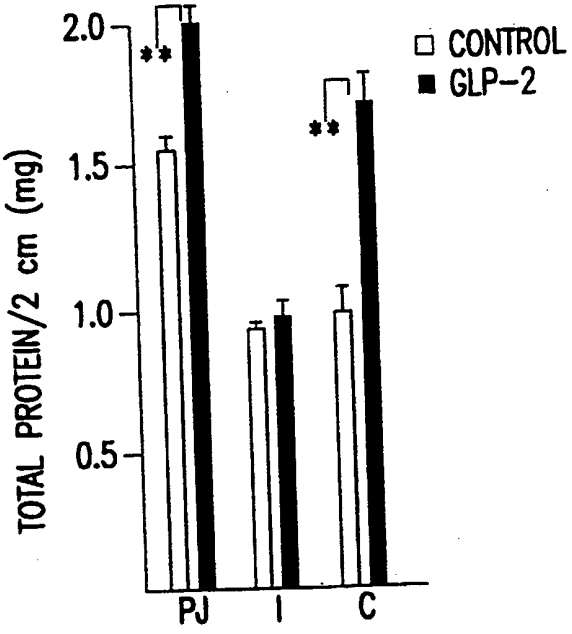

WEIGHT/2 cm SEGMENT (mg)
70
60
50
40
30
20
10
*
*
CONTROL
GLP-2
PJ
I
C
PJ
I
C
WET WEIGHT
DRY WEIGHT

FIG. 1A


TOTAL PROTEIN/2 cm (mg)
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1.5
1.0
0.5
**
**
CONTROL
GLP-2
PJ
I
C

FIG. 1B

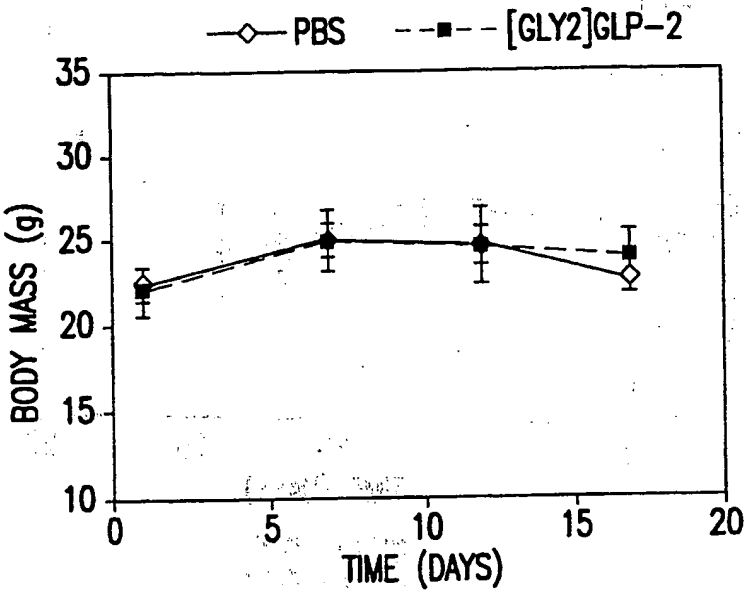
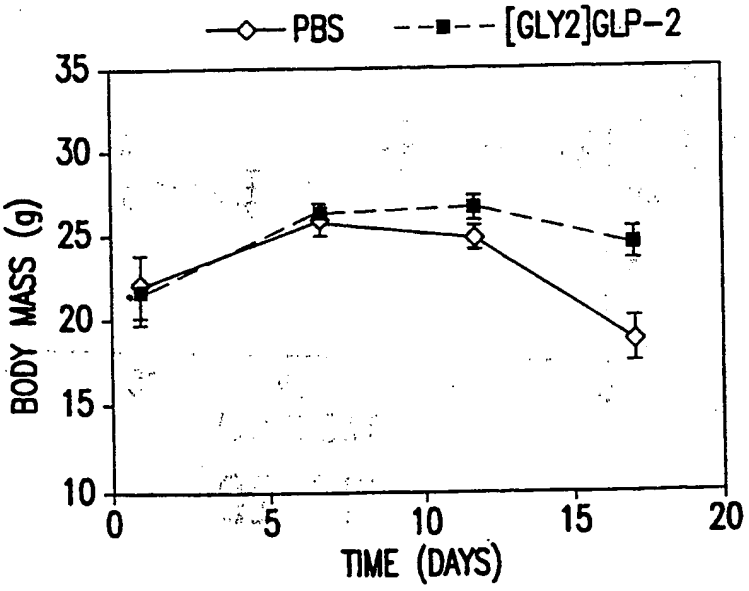

PBS
[GLY2]GLP-2
BODY MASS (g)
35
30
25
20
15
10
0
5
10
15
20
TIME (DAYS)

FIG. 2A


PBS
[GLY2]GLP-2
BODY MASS (g)
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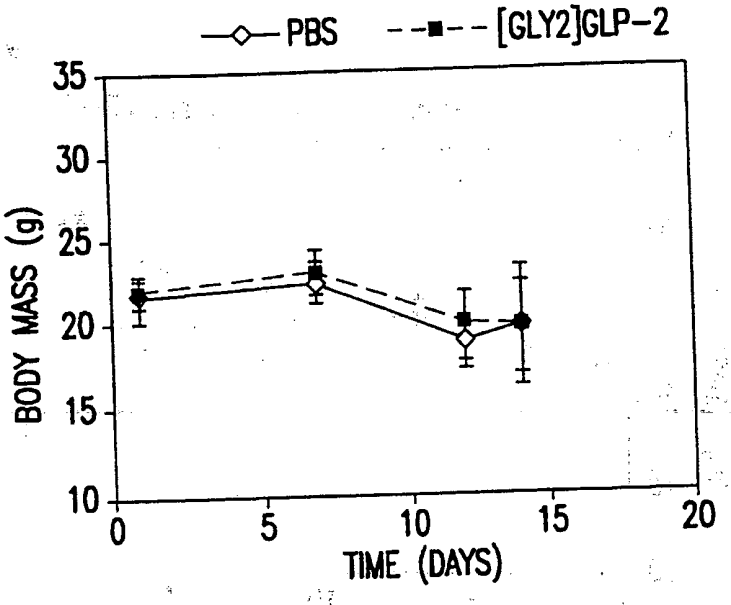
30
25
20
15
10
0
5
10
15
20
TIME (DAYS)

FIG. 2B


PBS
[GLY2]GLP-2
BODY MASS (g)
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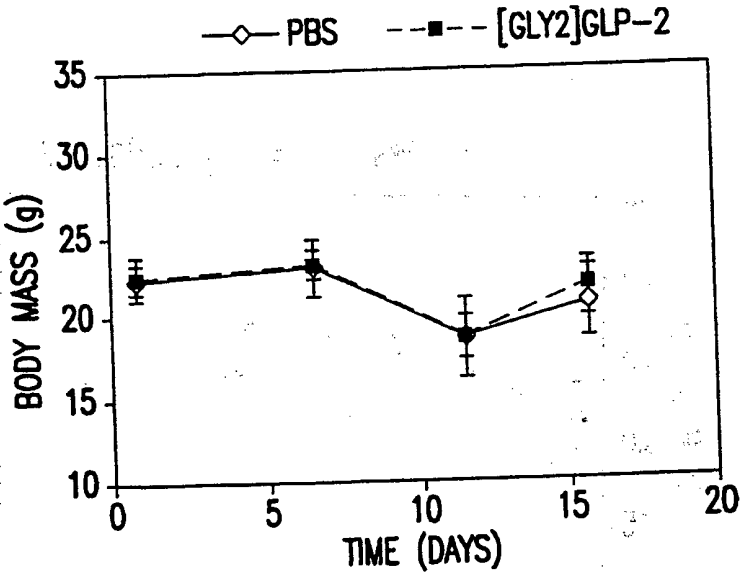
30
25
20
15
10
0
5
10
15
20
TIME (DAYS)

FIG. 2C


PBS
[GLY2]GLP-2
BODY MASS (g)
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30
25
20
15
10
0
5
10
15
20
TIME (DAYS)

FIG. 2D

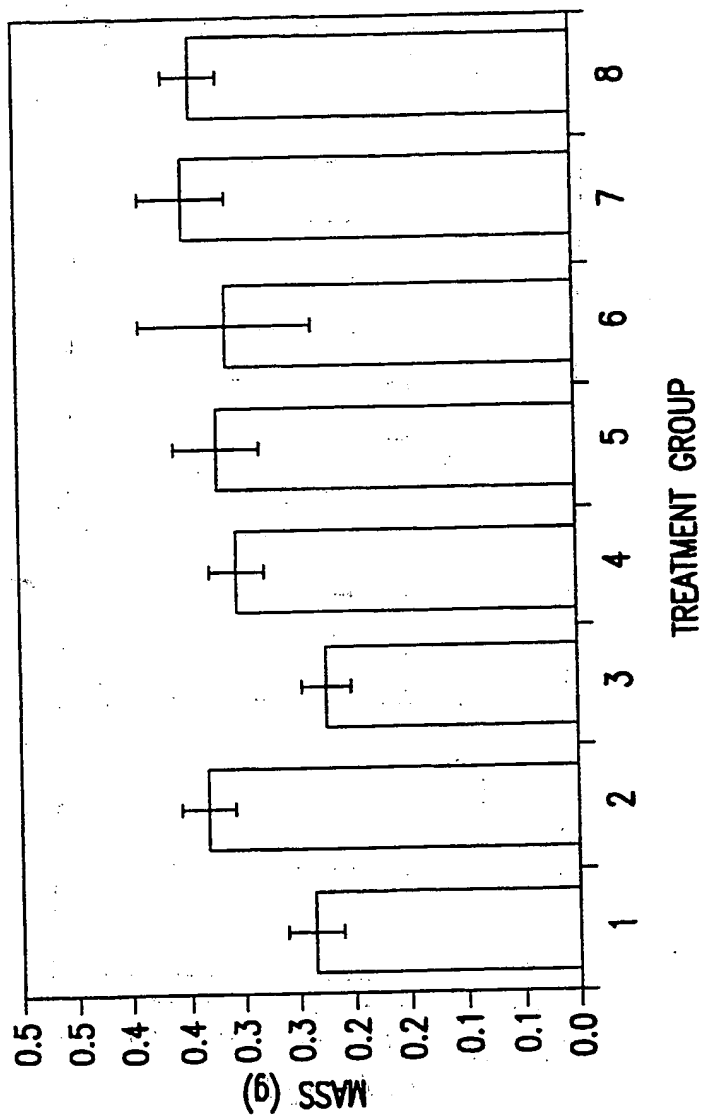

FIG. 3

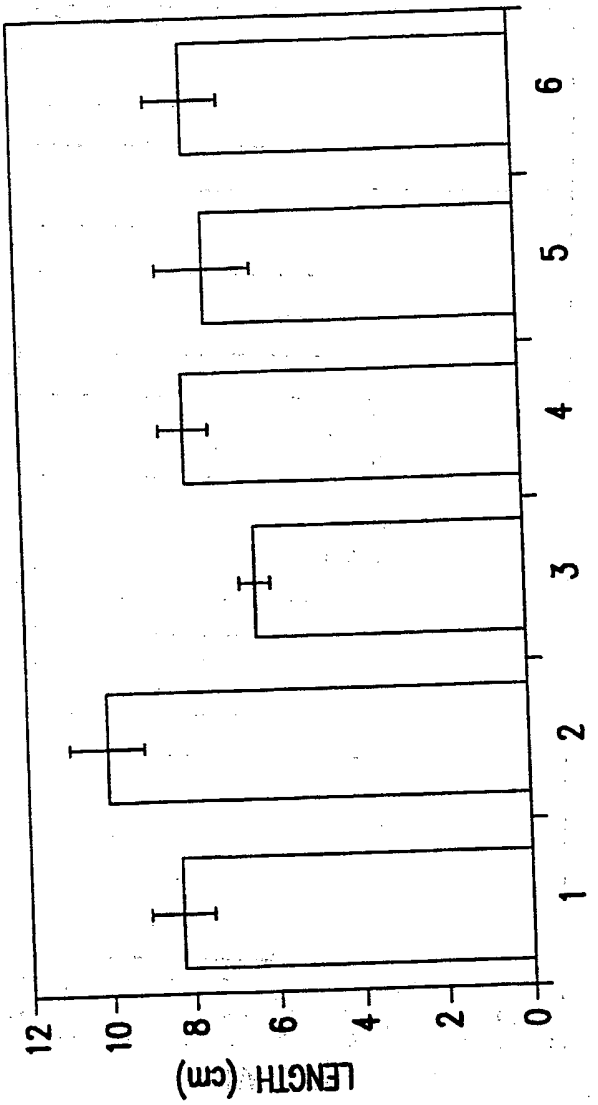

FIG. 4

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 98/00477

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/26 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and. where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	D.J. DRUCKER ET AL.: "INTESTINAL RESPONSE TO GROWTH FACTORS ADMINISTERED ALONE OR IN COMBINATION WITH HUMAN 'GLY2!GLUCAGON-LIKE PEPTIDE 2" AMERICAN JOURNAL OF PHYSIOLOGY: GASTROINTESTINAL AND LIVER PHYSIOLOGY, vol. 273, no. 6 PART 1, December 1997, pages G1252-G1262, XP002060300 WASHINGTON, DC, US	1-11,14
Y	see page G1252, right-hand column, paragraph 3 - page G1253, paragraph 1; figures 1,5,6 see page G1253, right-hand column, paragraph 5 see page G1257, left-hand column, paragraph 2 see page G1258, right-hand column, paragraph 2 - page G1259, left-hand column, paragraph 1 -/--	12,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 January 1999	16/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office. P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ryckebosch, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 98/00477

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C.-H. TSAI ET AL.: "INTESTINAL GROWTH-PROMOTING PROPERTIES OF GLUCAGON-LIKE PEPTIDE-2 IN MICE." AMERICAN JOURNAL OF PHYSIOLOGY: ENDOCRINOLOGY AND METABOLISM 36, vol. 273, no. 1, July 1997, pages E77-E84, XP002091514 WASHINGTON, DC, US see page E82, left-hand column, paragraph 1; figure 6C	1-7,11, 14
Y	see page E83, right-hand column, line 58 - page E84, left-hand column, paragraph 1	12,13
Y	WO 97 39031 A (ONTARIO INC. ET AL.) 23 October 1997 cited in the application see claim 21; example 4	12,13
E	WO 98 25644 A (ONTARIO INC.) 18 June 1998 cited in the application see page 4, line 28 - line 32 see page 11, line 2 - line 14 see page 17, disorders 15-17 see page 6, line 32 - line 34 see page 8, line 20 - line 26 see page 27; table III	1-11,14

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INTERNATIONAL SEARCH REPORT

ational application No.
PCT/CA 98/ 00477

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-11 and 14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

formation on patent family members

Inter ıal Application No
PCT/CA 98/00477

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9739031 A	23-10-1997	AU 2500297 A	07-11-1997
		US 5789379 A	04-08-1998
WO 9825644 A	18-06-1998	AU 5220098 A	03-07-1998